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Multiple layers of incompatibility to the parasitic witchweed, *Striga hermonthica*

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Summary

• Witchweeds (*Striga* spp.) are major agricultural pests that infest important crops in sub-Saharan Africa. *Striga hermonthica* parasitizes gramineous plants including sorghum, maize and rice, but not dicots. To understand host recognition mechanisms of *S. hermonthica*, we investigated its interaction with nonhost dicots including *Arabidopsis,* cowpea, *Lotus japonicus* and *Phtheirospermum japonicum*, a hemiparasite.

• *Striga hermonthica* seeds were pretreated with strigol, a germination stimulant, and allowed to germinate next to a potential host root. We characterized the histological phenotype of the interactions. Moreover, we monitored the infection of a host rice and the nonhost *P. japonicum* by *S. hermonthica* using time-lapse photography.

• All nonhost dicots tested did not support *S. hermonthica* shoot growth beyond the six leaf-pair stage; however, the arrest of parasite development occurred at different stages. *Striga hermonthica* haustoria were able to reach the steles of *Arabidopsis* and cowpea, while *L. japonicus* blocked *S. hermonthica* infection in the root cortex. *Striga hermonthica* often failed to penetrate *P. japonicum* roots.

• Our analysis indicates that there are at least four types of incompatible interaction to *S. hermonthica*. Combinations of these different incompatibility mechanisms contribute to the total resistance to *S. hermonthica*.

Introduction

Striga species, so-called witchweeds, are obligate root hemiparasites belonging to the *Orobanchaceae*, and represent the biggest weed threat to agriculture of sub-Saharan Africa. In particular, *Striga hermonthica* and *Striga asiatica*, which infect sorghum, maize, millet, and upland rice cause considerable yield losses (Aly, 2007; Ejeta, 2007; Scholes & Press, 2008). *Striga* species produce thousands of tiny seeds that remain viable in the soil for several decades. Thus the eradication of *Striga* seeds in the field is a laborious and difficult task.

Striga seeds remain dormant until they are exposed to host-derived germination stimulants called strigolactones (Shen *et al.*, 2006). For a long time it was a mystery why plants produce strigolactones which attract the noxious parasites. Recently, a role of strigolactones as a stimulant for hyphal branching of arbuscular mycorrhiza fungi was discovered (Akiyama *et al.*, 2005). Furthermore, strigolactones and/or their derivatives were identified as endogenous plant hormones to control shoot branching (Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008). These findings indicate that strigolactones are common compounds in higher plants, controlling plant architecture and symbiotic relationships, and which are exploited as recognition factors by the *Striga* parasites.

A germinated *Striga* seedling forms a specialized attachment and penetration organ called an haustorium in response to host-derived haustorium induction factors, which include various phenolic acids, quinones, and flavonoids (Keyes *et al.*, 2000; Yoder, 2001). The root tips of the parasite develop radial swelling and haustorial hairs that function as attachment anchors and penetration pegs (Keyes *et al.*, 2001). The swollen root portion contains a hyaline tissue (hyaline body) with characteristic dense cytoplasm and extracellular deposits (Dorr, 1997). After successful attachment and penetration of a host root, the parasite establishes vascular connections with its host and grows its own shoots, taking minerals and nutrients from the host plant.

One of the most efficient and cost-effective ways to control *Striga* infestations would be the development of resistance in host species. Extensive searches led to the identification of cultivars and wild relatives of several crop species, including sorghum,

maize and rice that were resistant to *Striga* parasitization (Albrecht *et al.*, 1999; Mohamed *et al.*, 2003; Rich *et al.*, 2004; Gurney *et al.*, 2006; Ejeta, 2007). The molecular/genetic basis for *Striga* resistance in resistant rice cultivars is beginning to be unravelled (Kaewchumnong & Price, 2008; Swarbrick *et al.*, 2008). Nevertheless, the resistance often is weak and tends to break down with the appearance of new parasite races (Aly, 2007; Rispail *et al.*, 2007).

The term 'nonhost resistance' describes the situation when all members of a plant species are resistant to all members of a pathogen species. Nonhost resistance is highly durable and effective, and is obviously the preferred state of resistant crops (Thordal-Christensen, 2003). Under natural conditions, *S. hermonthica* and *S. asiatica* infect gramineous but not dicotyledonous species (Musselman, 1980). Consequently, dicots are considered nonhosts for *S. hermonthica* and *S. asiatica*. Reports describing interactions of *S. hermonthica* or *S. asiatica* with dicots are limited. *S. asiatica* was reported to be able to penetrate roots of lettuce, marigold, and cowpea, but the infection becomes arrested in the root cortex (Hood *et al.*, 1998). A similar interaction between *Lotus japonicus* and *S. hermonthica* was reported by Kubo *et al.* (2008).

In this study, we investigated *S. hermonthica* interactions with nonhost species including *Arabidopsis thaliana*, cowpea (*Vigna unguiculata*), *L. japonicus* and the hemiparasitic *Phtheirospermum japonicum*, and compared these interactions with those between the parasite and rice cultivars that differed in *Striga* resistance (Gurney *et al.*, 2006). *S. hermonthica* haustorium formation and penetration attempts into rice and nonhost *P. japonicum* were investigated by time-lapse imaging. Our results suggest that there are at least four mechanisms of incompatible interaction to *S. hermonthica*.

Materials and Methods

Plant materials and growth condition

Striga hermonthica (Del.) Benth seeds collected from a sorghum field in 1994 in Kenya were provided by Dr A. G. Babiker (University of Khartoum, Sudan). Rice seeds (*Oryza sativa* L. subspecies *japonica*, cvs Koshihikari and Nipponbare) were obtained from NIBS (Tsukuba, Japan). Maize (*Zea mays* L., cv. Canbella-82) and cowpea (*Vigna unguiculata* (L.) Walp., cv. Akadane-Sanshakuohnaga) seeds were obtained from Takii Seed Corp. (Kyoto, Japan). *Phtheirospermum japonicum* (Thunb.) Kanitz seeds harvested in Okayama and Karuizawa, Japan were the kind gift from Dr T. Enomoto (Okayama University, Japan) and Dr H. Sato (Karuizawa botanical garden), respectively. *Arabidopsis* (accession Col-0), *L. japonicus* (ecotype Miyakojima MG-20) and *P. japonicum* seeds were surface-sterilized with 5%, 10% and 10% commercial bleach solution (approx. 6% sodium hypochloride; Kao, Tokyo, Japan) for 5 min, 5 min and 10 min, respectively. After thorough rinsing with water, the seeds were placed in full strength Murashige and Skoog (MS) media supplemented with 1% sucrose and kept at 4°C in the dark for 1–3 d for synchronized germination. *Arabidopsis* and *P. japonicum* seedlings were grown for 1 wk at 25°C (Fig. 1) or 22°C (Fig. 2) with 16-h light : 8-h dark cycles. Cowpea, maize and rice seeds were sterilized with 10% commercial bleach solution for 15 min and washed thoroughly with water. Seeds were placed on filter paper moisturized with sterile water. *L. japonicus*, cowpea and maize seedlings were kept in the dark at 26°C for 3 d and under 16-h light : 8-h dark cycles for an additional 4 d. Rice seedlings were grown in 16-h light : 8-h dark cycles at 26°C for 1 wk.

Striga hermonthica seed preconditioning and infection in a rhizotron system

Striga hermonthica seeds were briefly washed with 20% commercial bleach solution and sterilized in a renewed bleach solution for 5 min with gentle agitation. The seeds were then washed thoroughly with water and placed on glass fibre filter paper (Whatman GF/A) moisturized with sterile water. The sterilized seeds were preconditioned at 26°C in the dark for 10 d. Infection and subsequent observation were performed in a 'rhizotron' system as described by Gurney *et al.* (2006) with minor modifications. Square Petri dishes were filled with rockwool (Nichiasu, Tokyo, Japan) onto which nylon mesh was placed. The tops and bottoms of the Petri dishes were perforated to allow shoot growth and draining. Rice, cowpea and maize plants were grown in a larger rhizotron (24 × 24 cm square Petri dish and 100 μm nylon mesh), whereas *Arabidopsis*, *L. japonicus* and *P. japonicum* were grown in a smaller rhizotron (10 × 12 cm square Petri dish and 59 μm nylon mesh). Seedlings were transferred to the rhizotrons and fertilized with half-strength MS media. Rice, cowpea, maize and *L. japonicus* plants were kept in a glasshouse at temperature cycles of 28°C day : 20°C night with 12-h photoperiods. *Arabidopsis* and *P. japonicum* were grown either at 22°C or 25°C with 16-h light : 8-h dark cycles. After 2 wk in the rhizotron chambers, plants were inoculated with *S. hermonthica* seeds. Preconditioned *S. hermonthica* seeds were treated with 10 nM strigol (kind gift from Dr K. Mori; Hirayama & Mori, 1999) for 2–6 h and carefully placed next to roots of each host or nonhost plant. About 20 or 50 *S. hermonthica* seeds were inoculated with one plant in the smaller (*Arabidopsis*, *L. japonicum* and *P. japonicum*) or the larger rhizotron (maize, cowpea and rice). The rhizotron chambers were incubated under the same condition as described above, and developmental stages of *S. hermonthica* were examined after 2 wk and 4 wk.

Cross-sectioning and staining

To assess vascular connection, roots infected with *S. hermonthica* were randomly selected, segments were cut and fixed with ethanol–acetic acid (3 : 1) for 10 min under vacuum and stained with 1% Safranin-O (Wako Chemical, Osaka, Japan) solution

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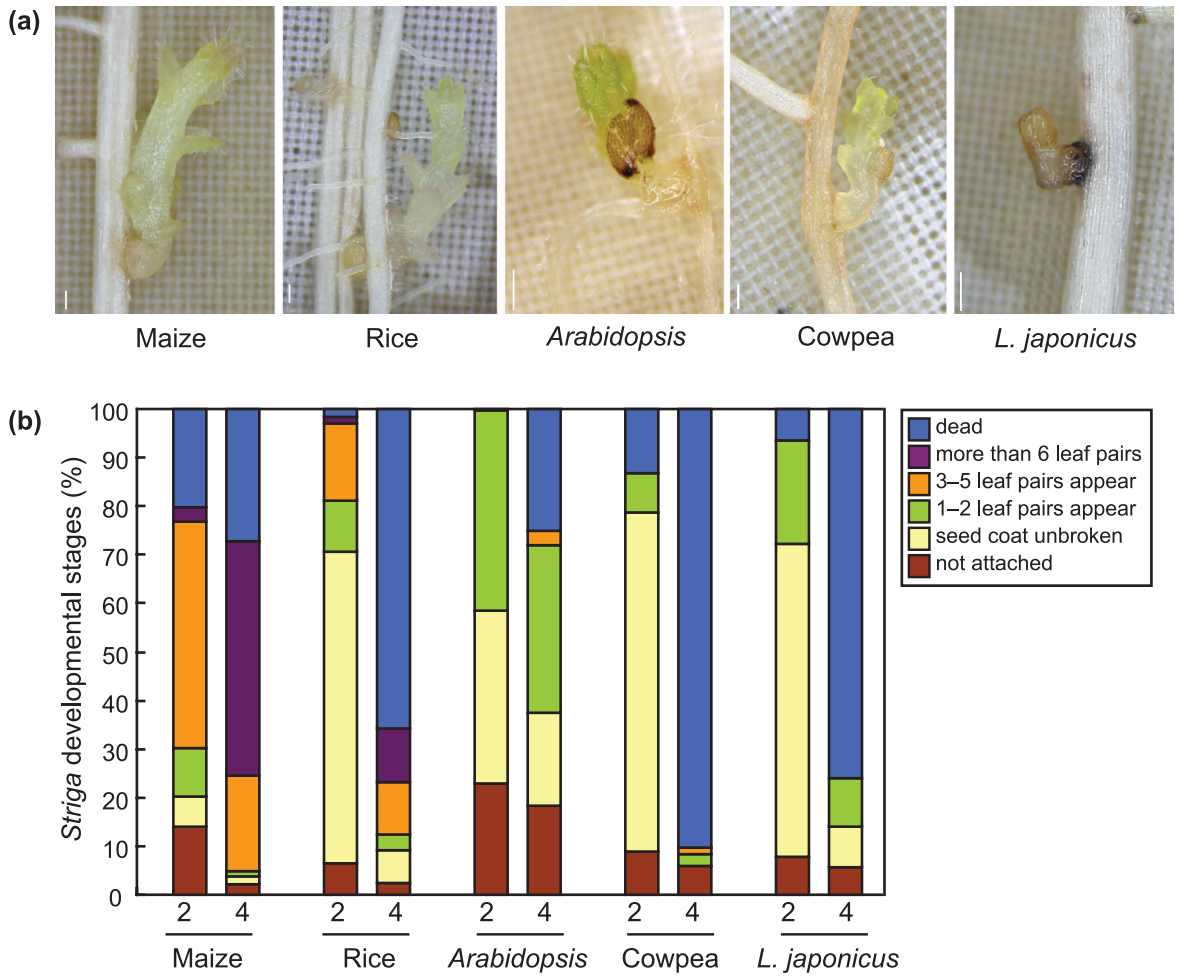


Fig. 1 Interaction between *Striga hermonthica* and maize, rice (cv. Koshihikari), *Arabidopsis*, cowpea and *Lotus japonicus*. (a) *S. hermonthica* parasitizing host or nonhost roots at 2 wk post inoculation (wpi). Bars, 200 μm. (b) Infection and development of *S. hermonthica* on host or nonhost roots. Developmental stages of *S. hermonthica* were determined at 2 wpi and 4 wpi. Bars show the percentages of *S. hermonthica* at each developmental stage relative to all *S. hermonthica* seeds co-cultivated with potential hosts; $n > 140$.

in 30% ethanol in a boiling water bath for 5 min. The roots were destained with chloral hydrate solution (2.5 g ml^{-1}) overnight with gentle agitation. For observation of cross sections, parasite-infecting roots were fixed in FAA (10% formaldehyde, 5% acetic acid, 50% ethanol) and embedded in Technovit 7100 (Heraeus Kulzer, Hanau, Germany) according to the manufacturer's instructions. Embedded samples were sectioned (4 μm thick) using a Leica microtome (RM2135), and sections were placed on AP-coated glass slides (Matsunami Glass, Osaka, Japan) and allowed to dry. The sections were stained with 1% Safranin-O in 30% ethanol, placed on a hot block until the solution had evaporated almost completely (3–5 min) and then washed thoroughly with water. The samples were dipped in a series of 95% ethanol with 0.5% picric acid, *c*. 0.01% ammonium, and 0.01% concentrated HCl for several seconds at each step. After washing with 100% ethanol, the samples were stained with 0.1% Fast Green (Wako Chemical) in 100% ethanol for 1 min and washed with excess ethanol. The double-stained sections were examined using a Keyence BIOZERO (Keyence, Osaka, Japan) microscope. With this staining technique, lignified tissues and secondary cell walls are stained red while the cytoplasm and nucleus are stained blue.

Time-lapse photography

Rice cv. Koshihikari and *P. japonicum* were grown for 10 d on a filter paper and for 2 wk in a rhizotron, respectively. The *S. hermonthica* seeds were preconditioned for 7–10 d and treated with 10 nM strigol for 16–20 h on a moist glass fibre filter paper to induce germination. Rice or *P. japonicum* plants were placed in a Petri dish and *S. hermonthica* seedlings were carefully located near their roots. Seedlings and roots were covered with a cover glass and 1% agarose in water to avoid dehydration. The Petri dishes were sealed with vinyl tape. Time-lapse photographs were automatically taken with a Keyence BIOZERO microscope at 30-min intervals for up to 44 h, and movie clips were created using the software Windows Movie Maker (Microsoft).

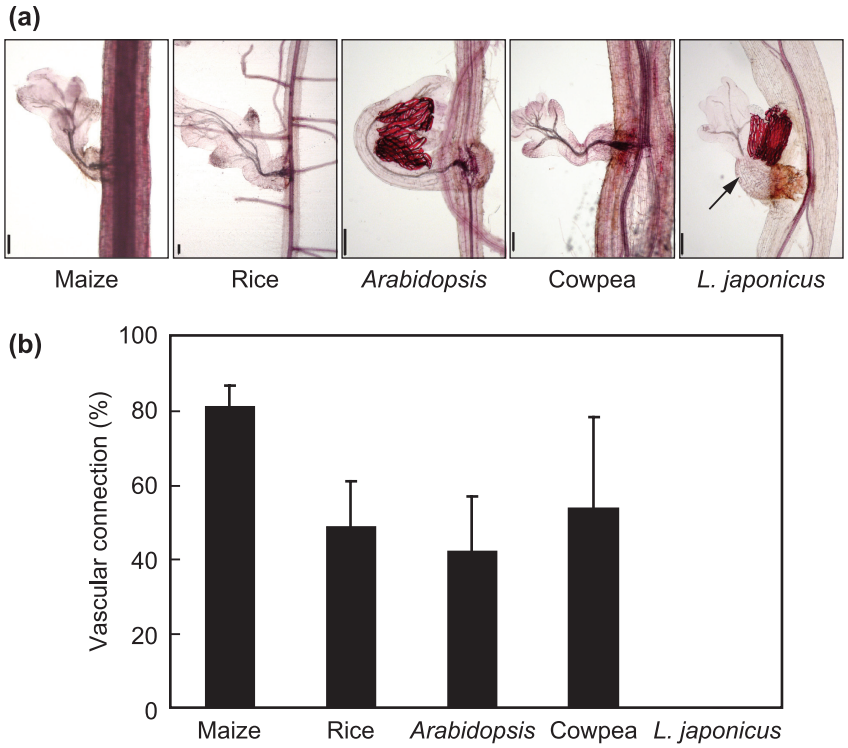


Fig. 2 Vascular connections between *Striga hermonthica* and host or nonhost roots. (a) *S. hermonthica* penetrating host or nonhost roots were stained with Safranin-O at 2 wk post inoculation (wpi). Arrow indicates *S. hermonthica* haustorium without vascular development. Bar, 200 μm. (b) The frequency of vascular connections was determined under a light microscope at 2 wpi and is expressed as the percentage of all *S. hermonthica* that had penetrated roots of a potential host. Data represent the mean and standard deviation of host/nonhost plants; n = 4 (maize), n = 14 (rice), n = 10 (*Arabidopsis*), 11 (cowpea) and n = 10 (*Lotus japonicus*).

Results

Interaction of *S. hermonthica* with host and nonhost plants

To observe *S. hermonthica* infection processes with host and nonhost plants, we employed an observation chamber rhizotron system (Gurney *et al.*, 2006). Maize and rice (cv. Koshihikari) were used as host plants and *Arabidopsis*, cowpea and *L. japonicus* as nonhost plants. *Striga hermonthica* seeds were pretreated with strigol for > 2 h to exclude effects of different germination ratios caused by different strigolactone productivities among species. Strigol-treated *Striga* seeds were placed next to the host or nonhost roots in the rhizotron chambers. Within a few days, > 90% of *S. hermonthica* seeds had germinated. Approximately 70–90% of the germinated seedlings initiated penetration into host or nonhost roots. We assessed developmental stages of *S. hermonthica* at 2 wk and 4 wk after inoculation (Fig. 1). Nearly 60% of *S. hermonthica* had developed shoots in co-cultivation with maize at 2 wk post inoculation (wpi). At 4 wpi, *c.* 45% of *S. hermonthica* had formed more than six leaf-pairs. In the case of rice, only 28% of *S. hermonthica* had developed shoots at 2 wpi, and 11% showed more than six leaf-pairs at 4 wpi. Although the parasite growth rates were different in maize and rice, *S. hermonthica* was able to continue development and eventually flowered in either case.

When co-cultivated with any of the nonhost plants *Arabidopsis*, cowpea and *L. japonicus*, no *S. hermonthica* plant had developed more than six leaf-pairs even at 4 wpi (Fig. 1a,b). A small proportion of *S. hermonthica* that had infected *Arabidopsis* and cowpea formed three leaf pairs, but failed to develop further. Similarly, *S. hermonthica* seedlings succeeded in penetrating *L. japonicus* root surfaces, but in most cases shoots failed to emerge from the seed coats, and none developed more than three leaf pairs. Accumulation of brown matter was observed at the contact points between *S. hermonthica* and *L. japonicus* roots (Fig. 1a). Our observations suggest that *Arabidopsis* and cowpea cannot completely prevent early phases of infection by *S. hermonthica* while *L. japonicus* aborts the *S. hermonthica* infection at early stages.

Vascular continuity in nonhost interaction

As *Arabidopsis* and cowpea supported early development of *S. hermonthica* shoots, it was obvious to enquire whether vascular connections were established in these nonhost interactions. We stained *S. hermonthica*-infected roots with Safranin-O to visualize lignified tissues such as xylem cells, and examined samples at 2 wpi (Fig. 2a). In maize and rice, *c.* 80% and 50%, respectively, of *S. hermonthica* vascular bundles were connected to host root vessels (Fig. 2b). In *Arabidopsis* and cowpea, vascular connections occurred at a similar frequency as in rice (Fig. 2b). However, no such connections were observed in *L. japonicus*, even when the *S. hermonthica* involved had developed one to two leaf-pairs (Fig. 2a). Thus, permanent infection by *S. hermonthica* was prevented through processes occurring after the establishment of vascular links in *Arabidopsis* and cowpea, but at earlier stages in *L. japonicus*.

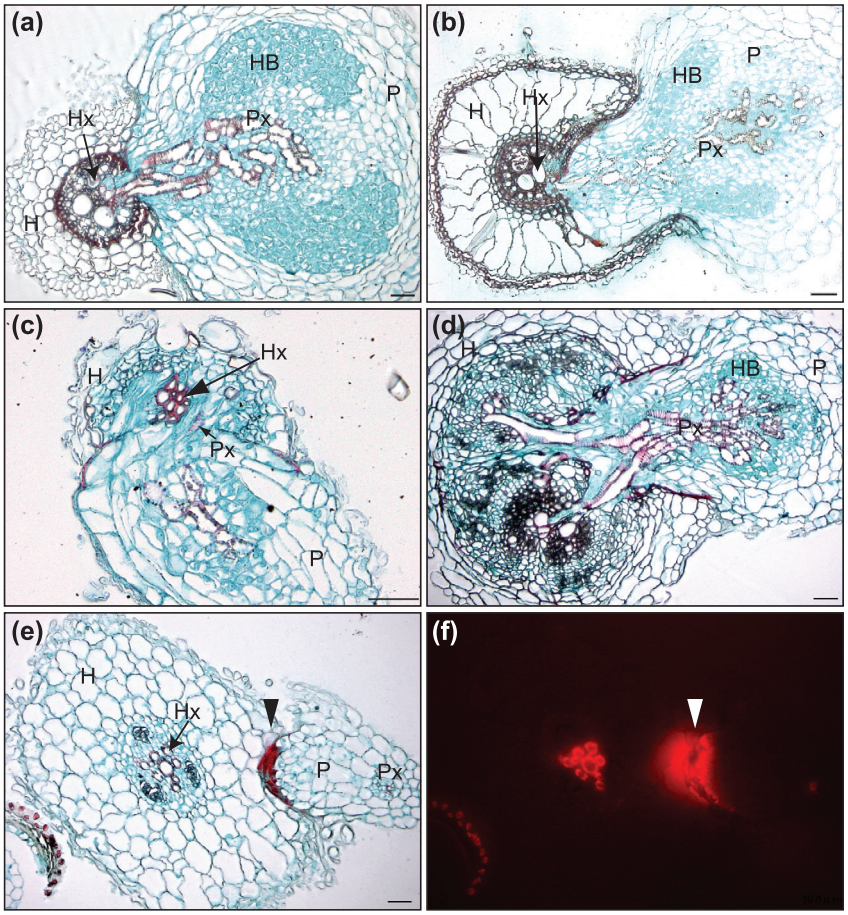


Fig. 3 Double-stained cross sections of *Striga hermonthica* haustoria penetrating host and nonhost roots. Technovit 7100-embedded tissues were cross-sectioned at 2 wk post inoculation (wpi) and stained with Safranin-O and Fast Green. (a) Maize, (b) rice (cv. Koshihikari), (c) *Arabidopsis*, (d) cowpea, (e) *Lotus japonicus*. (f) Fluorescence indicates Safranin-O-stained tissues. H, host or nonhost tissue; P, parasite (*S. hermonthica*); HB, hyaline body; Px, parasite xylem; Hx, host xylem. Bar, 50 μm.

To further characterize the interactions between *S. hermonthica* and nonhost plants, transverse sections of interacting tissues were examined after double staining with Safranin-O and Fast Green (Fig. 3). In roots of maize and rice, *S. hermonthica* parenchyma and xylem cells invaded the central cylinder and established vascular connections (Fig. 3a,b). Densely blue-stained cells represent the hyaline body which indicates successful parasitization. In *Arabidopsis* and cowpea roots, parasite parenchyma and vessel cells invaded the nonhost tissue but failed to penetrate vessel elements (Fig. 3c,d). In *L. japonicus*, the parasite invasion was blocked in the root cortex; neither parasite vessel cells nor a hyaline body were observed in the haustorium at 2 wpi (Fig. 3e). Safranin-O stainable substances accumulated at the interface between *S. hermonthica* and *L. japonicus* (Fig. 3e,f, arrowheads) and were observed at 1 wpi when the *S. hermonthica* endophytes still retained dividing cells (see the Supporting Information, Fig. S1). This suggests that the accumulation of these substances in the contact zones had begun before the penetration process had come to a halt.

Different susceptibilities between rice cultivars

Gurney *et al.* (2006) reported that the rice cultivar Nipponbare is resistant and the other cultivars, including Koshihikari, are susceptible to *S. hermonthica* parasitism. We investigated the difference between cultivars Nipponbare and Koshihikari in our system. In Koshihikari, *c.* 10% of *S. hermonthica* developed more than six leaf-pairs at 4 wpi and continued to grow until flowering (Fig. 4a). Conversely, *S. hermonthica* infecting Nipponbare developed more than six leaf-pairs at a significantly lower rate (approx. 2.5%) at 4 wpi. However, at 2 wpi, the frequency of shoots with one to two leaf pairs was not significantly different between *S. hermonthica* plants parasitizing Koshihikari or Nipponbare (Fig. 4a). Interacting host and parasite tissues were stained with Safranin-O and the frequency of vascular connections was determined (Fig. 4b). Nipponbare showed fewer vascular connections but when analysed by Student's *t*-test, the difference proved not to be significant ($P > 0.1$). *Striga hermonthica* plants that had developed one to two leaf-pairs on Nipponbare were checked and successful invasions of the host stele were found as early as 1 wpi (Fig. 4c, upper panels). However, we often observed that *S. hermonthica* that failed to grow shoots in Kohishikari did not penetrate the host's endodermis (Fig. 4c, lower panels). This phenotype was reminiscent of that reported for the cv. Nipponbare resistance phenotype (Gurney *et al.*, 2006). Those results suggest that the difference in susceptibility between Koshihikari and Nipponbare is not only caused by their different abilities to prevent *S. hermonthica*

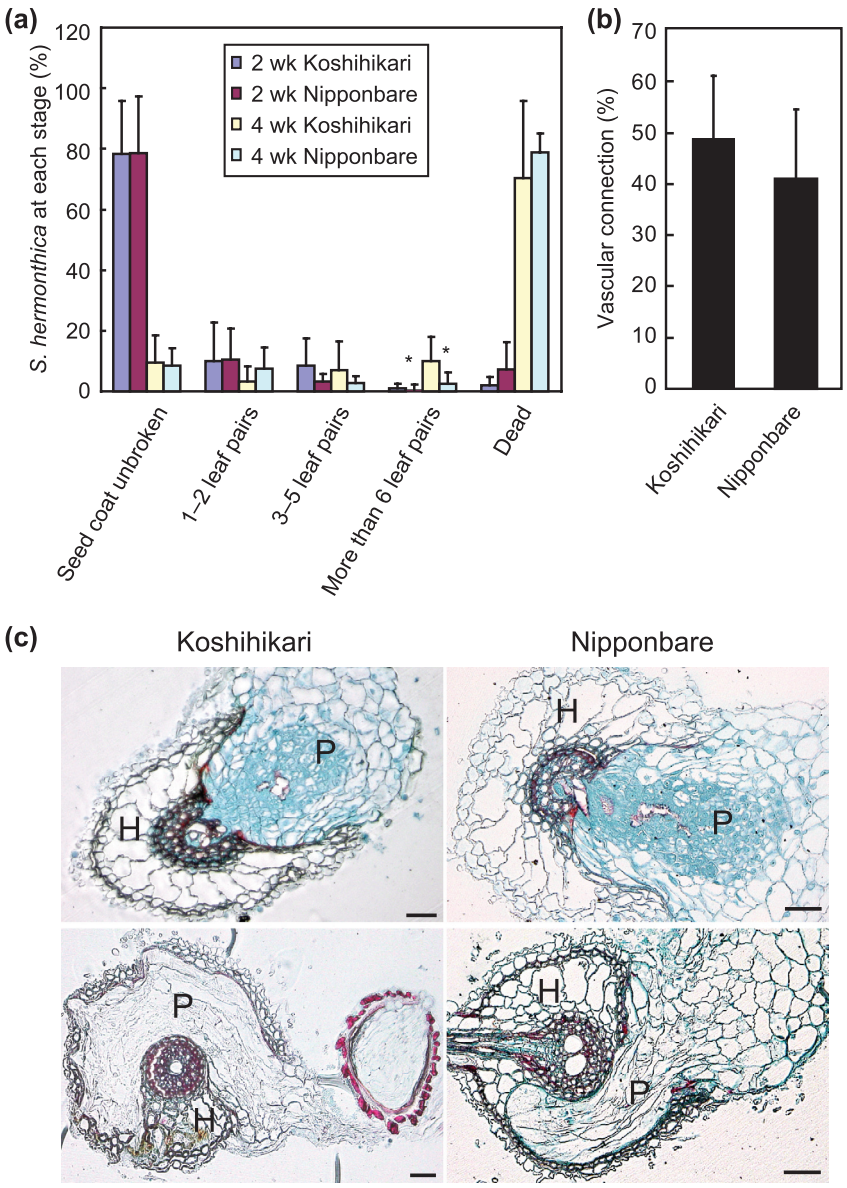


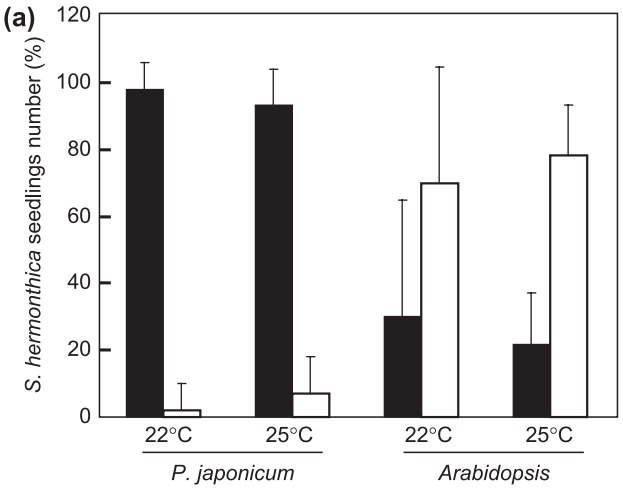
Fig. 4 Infection rates of *Striga hermonthica* on roots of two rice cultivars, Koshihikari and Nipponbare. (a) Infection rates of *S. hermonthica* at 2 wk and 4 wk post inoculation (wpi). Frequencies of developmental stages are expressed as percentages of parasites that had penetrated host tissues. Asterisks indicate significant differences between the two rice cultivars ($P < 0.06$). Data represent means with a standard deviation of seven individual host plants. (b) Vascular connectivity between *S. hermonthica* and rice cv. Koshihikari and cv. Nipponbare was determined after whole-root safranin staining at 2 wpi. Data represents the mean and standard deviation; $n = 14$ (cv. Koshihikari), $n = 15$ (cv. Nipponbare). (c) Cross-sections of *S. hermonthica* penetrating a root of rice cv. Koshihikari (left) and cv. Nipponbare (right) with successful vascular connection at 1 wpi (upper panels) and those failing to connect to vascular tissues at 4 wpi (lower panels). Bars, 50 μm.

from penetrating the root endodermis, but also by post-vascular connection resistance expressed in cv. Nipponbare.

Interaction of *S. hermonthica* with the hemiparasitic plant *P. japonicum*

All host and nonhost plants analysed induced development of *S. hermonthica* haustoria and were susceptible to parasite penetration. We tested whether *S. hermonthica* was capable of infecting other parasitic members of the Orobanchaceae. The facultative hemiparasite *P. japonicum*, which is distributed over East Asia including Japan, was chosen and co-cultivated with rice, maize and cowpea for 5 wk. We found lateral haustorium formation and penetration of *P. japonicum* roots into the roots of all three potential hosts (Fig. S2a). The germination of *P. japonicum* did not require strigolactone and the root exudate of *P. japonicum* was able to stimulate germination of *S. hermonthica* (Fig. S2b).

When *S. hermonthica* seeds were co-cultivated with *P. japonicum* in rhizotrons, > 90% of the *S. hermonthica* seedlings failed to penetrate potential host roots at 2 wpi (Fig. 5a). The ratio of penetration was slightly higher at 25°C than at 22°C; this tendency was also confirmed when *Arabidopsis* was the potential host. Curiously, even when *S. hermonthica* roots were in physical contact with *P. japonicum* roots, they failed to initiate penetration and continued to elongate (Fig. 5b). In this case, the root length of *S. hermonthica* became longer than the average length of roots that successfully penetrated rice roots (Fig. S2c), indicating that the failure of penetration was not caused by growth inhibitory effects of *P. japonicum* roots. At very few



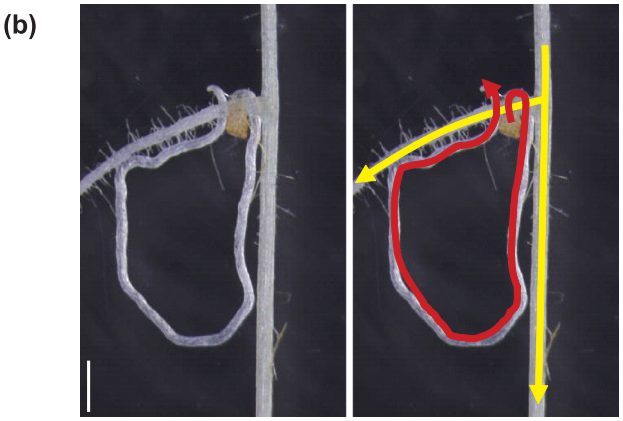


Fig. 5 *Striga hermonthica* often fails to penetrate roots of the hemiparasitic *Phtheirospermum japonicum*. (a) Rates of successful penetration of *P. japonicum* and *Arabidopsis by S. hermonthica* at the temperatures indicated. $n > 11$: closed bars, not penetrated; open bars, penetrated. (b) A representative case of S. hermonthica failing to penetrate a *P. japonicum* root. In the right panel, roots of *P. japonicum* and *S. hermonthica* are marked by yellow and red arrows, respectively. Bar, 200 μm.

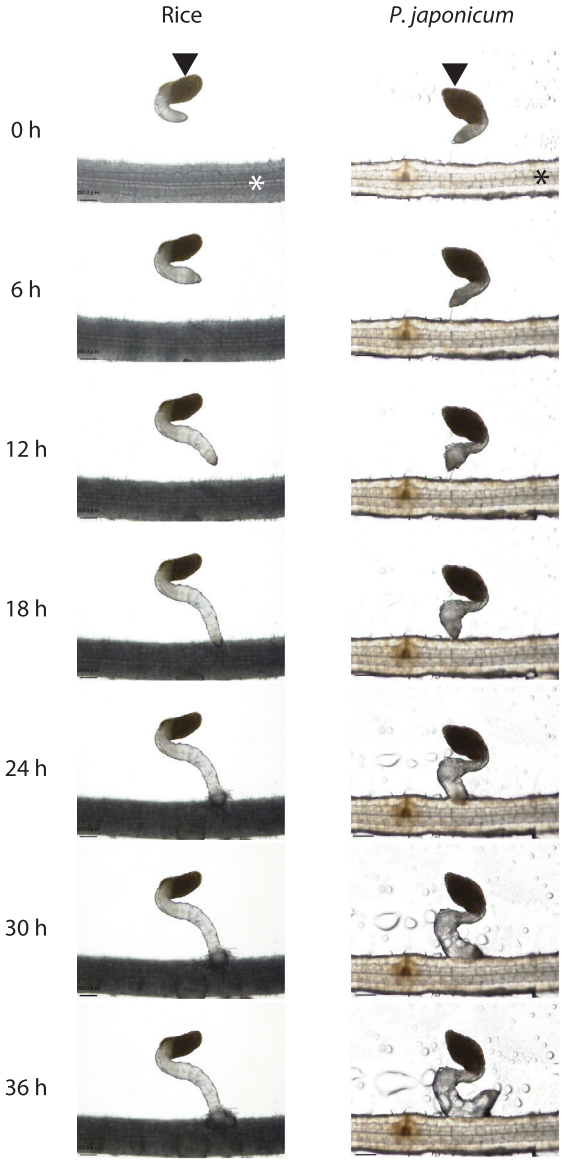


Fig. 6 Time-lapse image series showing the interactions of *Striga hermonthica* with rice and *Phtheirospermum japonicum*. Germinated *S. hermonthica* seedlings (arrowheads) were placed close to rice (left panels, white asterisk) and *P. japonicum* (right panels, black asterisk) roots, and their growth behaviour was monitored over 36 h. Bar, 200 μm.

occasions, *S. hermonthica* succeeded to penetrate *P. japonicum* roots. In these rare cases, growth of the *S. hermonthica* endophyte was restricted to the *P. japonicum* root's cortical cell layers, and safranin-stainable substances accumulated in the contact zones of *P. japonicum* and *S. hermonthica* (Fig. S2d).

Time-lapse observation *in vitro* of the infection of rice and *P. japonicum* roots by *S. hermonthica*

To understand how *S. hermonthica* reacts to the close proximity of *P. japonicum* roots, we recorded a time-lapse photographic series of the interaction. As a control, *S. hermonthica* seedlings were placed near a host rice root and a series of photographs were taken at 30-min intervals (Fig. 6, Video S1). At 6 h of co-incubation, the *S. hermonthica* root slightly swelled and changed the growth direction toward the rice root. The *S. hermonthica* root tip touched the rice root within 18 h of observation, and proliferation of haustorial hairs and root tip swelling progressed synchronously. The haustorium volume continued to increase during the process of penetration until the end of observation. Similarly, *S. hermonthica* root tips initially turned toward the *P. japonicum* root at 6 h (Fig. 6, Video S2). However, in contrast to the interaction with rice, there were no symptoms of haustorium differentiation after the roots had come into direct contact at 18 h. Eventually, the *S. hermonthica* root tips turned away.

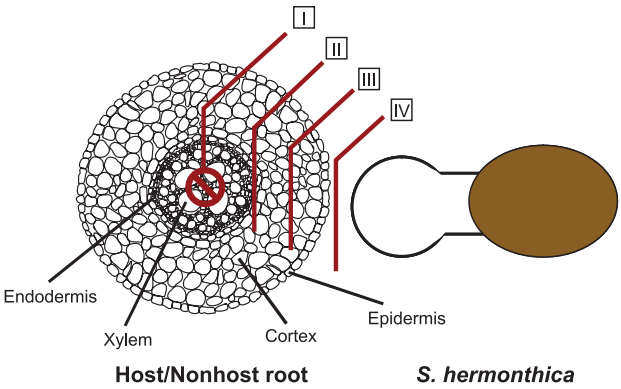


Fig. 7 Schematic illustration of incompatible interactions between *Striga hermonthica* (right) and host or nonhost plants (left, shown as a transverse section of a root). Four layers of incompatibility described in the text are presented. I, Incompatibility expressed after vascular connection, which was observed in *Arabidopsis*, cowpea and in rice cv. Nipponbare. II, Endodermis blockage, which is observed in rice cv. Nipponbare as well as in cv. Koshihikari. III, mechanical barrier in the root cortex, observed in interaction with *Lotus japonicus* and occasionally with *Phtheirospermum japonicum*. IV, incompatibility preventing attachment, observed in interaction with *P. japonicum*.

These results indicate that *S. hermonthica* does not properly recognize *P. japonicum* roots as potential hosts most of the time.

Discussion

In this study, we examined the interaction between *S. hermonthica* and various host and nonhost species. There are at least four types of incompatible interactions that can be distinguished by the host root cell layer at which invasion stops, for example: layer I, after vascular connection; layer II, at endodermal cell layers; layer III, at cortex cell layers; and layer IV, before *S. hermonthica* attachment (Fig. 7).

Interaction with nonhosts *Arabidopsis* and cowpea (layer I)

Arabidopsis and cowpea are nonhost plants for *S. hermonthica* as the parasite is unable to complete its life cycle with these species. However, the frequencies of root penetration and vascular connection in *Arabidopsis* as well as cowpea do not significantly differ from that observed in rice cv. Koshihikari, a susceptible host plant. Thus, the incompatibility of *S. hermonthica* with *Arabidopsis* and cowpea must result from mechanisms that take effect mainly after the establishment of vascular connections (Fig. 7, layer I). This phenotype is similar to that found in the wild relative of maize, *Tripascum dactyloides*, which exhibits resistance to *S. hermonthica* often associated with the immature development of a hyaline body, a characteristic tissue for parasitic plant haustorium, with densely stained cells encircling parasite vascular core (Gurney *et al.*, 2003). In *T. dactyloides*, the xylem of the parasite connects to the host xylem cells. While we observed hyaline bodies in haustoria in cowpea and *Arabidopsis* roots, these bodies were less well developed than those found in the natural hosts, maize and rice. *Striga hermonthica* parenchyma cells were in physical contact with the xylem in cowpea and *Arabidopsis*, but no penetration of vessel elements was observed. This is a marked contrast to the interaction with *T. dactyloides* (Gurney *et al.*, 2003).

Hood *et al.* (1998) reported that 80% of *S. asiatica* infections stopped in the root cortex in cowpea, and no vascular connections were observed 12 d post inoculation. We found that the vasculature of *S. hermonthica* reached cowpea steles at 2 wpi in about half of all cases. These different rates of successful penetration may be because of differences between species, infection methods, or timing of observation. In any case, our results indicate that the cortex is not the last barrier for *S. hermonthica* in cowpea roots; rather, the parasitization cannot be considered successful even after the connection of the vasculatures. Therefore, the incompatibility between cowpea and *S. hermonthica* results from at least two mechanisms, one acting in the root cortex and one after the formation of vascular connections.

Interaction with different rice cultivars (layer I and II)

Gurney *et al.* (2006) reported that the Nipponbare cultivar of rice is resistant mainly because of a failure to establish xylem–xylem connections between host and parasite (Fig. 7, layer II). Although our result confirmed the different susceptibilities of cvs Koshihikari and Nipponbare reported by Gurney *et al.* (2006), the characteristic incompatible phenotype in which the parasite endophyte fails to penetrate the endodermal layer was often observed even in the susceptible cultivar Koshihikari. The frequency of vascular continuity between host and parasite does not significantly differ between Koshihikari and Nipponbare, suggesting that resistance in Nipponbare is caused not only by an inhibition of endodermis penetration (Fig. 7, layer II) but also by mechanisms that act after vascular connections have become established (Fig. 7, layer I). Previous rhizotron experiments showed that a small fraction of *S. hermonthica* parasitizing Nipponbare had developed three to five scale leaf-pairs at 9 d after infection (Gurney *et al.*, 2006). In these cases, vascular connections probably had formed. By contrast, pot infection experiments showed that if an emergence of aerial organs of *S. hermonthica* growing on cv. Nipponbare occurs, it does so > 30 d later than in the most susceptible rice cultivar IR64 (Kaewchumnong & Price, 2008). Although these data were obtained under different conditions and should be compared with care, they appear in to be accord with the notion that resistance in cv. Nipponbare includes factors that act after the establishment of vascular connections and delay the development of the parasite.

Interaction with a nonhost *L. japonicus* (layer III)

When *S. hermonthica* infects *L. japonicus*, penetration comes to a halt in the root cortex and safranin-stainable substance(s)

accumulate in the contact zone (Fig. 7, layer III). This observation is reminiscent of the dense toluidine blue staining that has been observed at the interface between *S. hermonthica* and *L. japonicus* tissues (Kubo *et al.*, 2008). The accumulation of specific substances at the interface between a parasite and an incompatible host has been suggested repeatedly to be related to a resistance mechanism (Maiti *et al.*, 1984; Arnaud *et al.*, 1999; Goldwasser *et al.*, 1999; Cameron *et al.*, 2006; Echevarria-Zomeno *et al.*, 2006; Perez-de-Luque *et al.*, 2006). For example, *Orobanche crenata* penetration was blocked in the root cortex of a resistant pea, and strong staining with Safranin-O of the host–parasite interface together with host defence responses such as accumulation of H_2O_2 and callose or cell wall crosslinking was often observed (Perez-de-Luque *et al.*, 2006). The incompatible interaction between *O. cumana* and sunflower includes the accumulation of phenolic compounds (Echevarria-Zomeno *et al.*, 2006). Cameron *et al.* (2006) reported that the penetration structure of the hemiparasite *Rhinanthus minor* was encapsulated in a darkly stained layer, possibly related to lignification, in the nonhost forb *Leucanthemum vulgare*. In the interaction between *S. hermonthica* and the resistant sorghum Framida, the parasite reaches the host stele while phenolic substances accumulate in and around the host central cylinder (Arnaud *et al.*, 1999). In the present study, *S. hermonthica* was unable to penetrate *L. japonicus* steles. The safranin-stainable substance(s) accumulated at 1 wpi when active penetration was evident. These observations support the notion that the accumulating substances may act as a mechanical barrier or encapsulation structure that prevents the progression of parasite invasion.

Interaction with a hemiparasitic plant *P. japonicum* (layer III and IV)

All nonhost plants tested in this study were susceptible to penetration by *S. hermonthica*, although the penetration efficiency differed. Our results are consistent with the previous conclusion that the haustorial initiation, attachment and penetration processes are generally nonspecific and occur in nonhost as well as host plants (Hood *et al.*, 1998). However, our study indicates that attachment and penetration of *P. japonicum* roots by *S. hermonthica* occur at a significantly lower frequency than in other nonhost plants, suggesting that *P. japonicum* inhibits *S. hermonthica* attachment (Fig. 7, layer IV). Time-lapse photography showed that *S. hermonthica* roots grew toward *P. japonicum* roots in their vicinity, but failed to form haustoria and ultimately turned away. The active forms of haustorium-inducing quinones exist only in restricted redox potential ranges (Keyes *et al.*, 2000). *Striga asiatica* roots produce H_2O_2 to oxidize and activate quinones produced by cell wall degradation. Exogenous application of catalase inhibits haustorium induction by 2,6-dimethoxybenzoquinone in *S. asiatica* (Kim *et al.*, 1998). The roots of *P. japonicum* may secret reducing components or catalase to prevent haustorium initiation. *In vitro* experiments showed that the irreversible commitment to haustorium development requires several hours of exposure to haustorium-inducing factors, and that the length of the exposure period required is dependent on the amounts of haustorium-inducing factors (Yoder, 2001). Thus, as an alternative possibility, *P. japonicum* roots may produce smaller amounts of haustorium-inducing factor(s) than other nonhost plants, or may produce factors of low stability.

The formation of haustoria in roots of the same species or of the same plant (autoparasitism) was described in the root parasite *Alectra vogelii* in the family *Orobanchaceae* (Nwoke, 1982) as well as in species of *Cuscuta* (Jacob *et al.*, 1985). Autoparasitism also occurs infrequently in mistletoes (Ehleringer & Schulze, 1985). Similar to the situation where *S. hermonthica* penetrate *P. japonicum* roots, a safranin-stainable substance accumulates in contact zones in the autoparasitism of *A. vogelii* (Nwoke, 1982). This leads to the following interpretation. *Phtheirospermum japonicum* blocks *S. hermonthica* infection in two steps: first, it inhibits attachment and second, it blocks further penetration in the root cortex (Fig. 7, layer III) by a mechanism that resembles that of autoparasitism avoidance. Therefore, a better understanding of autoparasitism avoidance on the molecular level may facilitate the development of strategies to improve the resistance of crops against *Striga* parasites.

Conclusion

Four types of incompatibilities between *S. hermonthica* and various host and nonhost plants were characterized. It is noteworthy that incompatibility phenotypes may vary in seed populations. For example, a small portion of *S. hermonthica* can penetrate *P. japonicum* roots, although the majority is prevented from penetration. Similarly, half of *S. hermonthica* plants stopped growing in the root cortex of cowpea while the other half died after having established vascular connections. These findings may reflect genetic variability of *S. hermonthica*, which is an obligate outcrossing plant. This feature of *S. hermonthica* may make it difficult to generate completely resistant host plants. However, as nonhost plants successfully prevent *S. hermonthica* from completing its life cycle, a combination of incompatibility mechanisms at several stages works effectively for *Striga* resistance.

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Supporting information

Additional Supporting Information may be found in the online version of this article.

Fig. S1 Cross section of the contact zone between *Lotus japonicus* and *Striga hermonthica* at 1 wk post inoculation (wpi).

Fig. S2 Host specificity of *Phtheirospermum japonicum*, and *Striga hermonthica* infection of *P. japonicum*.

Video S1 Time-lapse video image of *Striga hermonthica* infection to a rice root.

Video S2 Time-lapse video image of *Striga hermonthica* co-cultivated with a *Phtheirospermum japonicum* root.

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